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The Commissioner of Patents

BY FACSIMILE
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30 November 2004

URGENT
RESPONSE TO WRITTEN OPINION
DUE: 30 NOVEMBER 2004

Madam

IN THE MATTER OF International Patent Application No. PCT/AU2003/001118 in the name of COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION
Entitled METHODS FOR THE CHEMICAL AND PHYSICAL MODIFICATION OF NANOTUBES, METHODS FOR LINKING THE NANOTUBES, METHODS FOR THE DIRECTED POSITIONING OF NANOTUBES, AND USES THEREOF
Our Ref: DAB:AMM:FP18328

We refer to a Written Opinion dated 29 April 2004 and enclose new pages 3, 4, 5, 5a, 105, 106 and 107 to replace pages 3, 4, 5, 105, 106 and 107 at present on file. A working copy of these pages with hand-written amendments is also enclosed for your reference.

In response to Sections V and VI of the Opinion, we propose to only deal with references D1 to D6 as they were either published or possess an earlier priority date than the present application. We will now discuss each of these references in turn.

D1

This paper describes a method for the non-covalent functionalisation of single-walled carbon nanotubes. They focus on non-covalent attachment because they do not want to change the sp2 structure of carbon, reasoning that this is necessary to preserve the nanotubes' electronic properties. Their method involves physical adsorption of 1-pyrenebutanoic acid, succinimydyl ester, onto the nanotubes, producing "succinimydyl ester groups that are highly reactive to nucleophilic substitution by primary and secondary amines that exist in abundance on the surface of most proteins".

As this paper does not discuss the chemical or photochemical attachment of any biomolecule to nanotubes, it is not relevant to the present methods of chemically or photochemically attaching nucleic acid molecules to nanotubes. With regard to the physical adsorption method of attachment. This paper only teaches how to attach proteins, not DNA. Further, it would not

be obvious to a person skilled in the art from this paper as to how single-stranded DNA could be attached in the manner described and be functional for the following reasons:

(i) the attachment via amide bond formation using an amine group on the DNA could occur through amines on the bases, thus preventing the subsequent binding of the DNA strand of complementary base sequence.

(ii) if the attachment did occur through a 5' or 3' terminal linker on the single-stranded DNA, parts of the DNA strand could still be strongly adsorbed on the nanotube's surface, and hence would be unable to bind the DNA strand with a complementary base sequence.

D2

This paper describes a double-stranded DNA molecule (a self-complementary 14 bp double helix, bound to cis-platin ($cis[Pt(NH_3)_2]^{2+}$)) physically adsorbed on the surfaces of multi-walled nanotubes. The DNA was indirectly visualised on the nanotubes by imaging the Pt using TEM. DNA was reported to be strongly bound, and the thickness of the adsorbed layer is consistent with a DNA double helix lying along a surface in a vacuum.

In this paper, the DNA is not functional in the sense that the double helix lying along the surface cannot be used to bind a complementary single strand and do work such as directing the assembly of the nanotubes or linking them etc. It is also unlikely to be able to attract a DNA-binding protein.

Even if this experiment was done with a single strand of DNA, the strand would lie along the surface and hence be unable to bind another DNA strand of complementary base sequence. Thus the method does not teach how to adsorb functional DNA molecules on the nanotubes.

D3 .

Although this reference discloses DNA attachment by a variety of chemical means, there is no reduction to practice regarding DNA, only proteins. It is not obvious that functional DNA could be attached using these methods, as detailed above for D1. D3 describes a possibility for DNA attachment on page 83, but this is through a protein link: nanotubes – biotin – streptavidin – biotin – DNA.

There is no description of the photochemical attachment of proteins or DNA and there is no mention of using vertically aligned nanotubes.

D4

D4 relates to carbon nanotubes functionalised on the sidewalls through non-covalent methods (such as physical adsorption, stacking, hydrophobic interactions etc).

This reference focuses on functionalisation of nanotubes with proteins, with mention of DNA once only in the description on page 5 and claim 13. There is no specific teaching regarding DNA.

The approach for functionalising nanotubes by the method described in D4, is that the surface of the nanotube must be totally covered by the physically-adsorbed anchor to which the protein or DNA is to be chemically attached. Otherwise the protein or DNA may be physically adsorbed to those areas of the nanotubes which were not covered by the anchor. The difference could not be identified unless checked and this was not done in D4. It is very hard to totally coat the surfaces of so-called "dispersed" nanotubes which usually exist in small bundles. It is far easier to coat the surfaces of nanotubes physically held apart

For a protein, it probably would not make much difference whether the protein was physically adsorbed directly to the surface of the nanotube, or indirectly through a physically-adsorbed anchor, because the 3-D structure of the protein would be retained due to the interactions between the amino-acids. The functionality of each particular protein would depend on whether or not the protein's receptor site is exposed to solution, or is face down on the nanotube surface. There is no information (eg XPS data) in D4 to indicate that their anchors are bound to the nanotubes' surfaces and/or that proteins are chemically attached to these anchors. It is quite possible that the proteins are not chemically attached to the anchors, but are directly adsorbed on the nanotubes' surface side-by-side with the anchors.

When attaching DNA to nanotubes, some effort is required to prevent the DNA from lying down on the surface of the nanotube and becoming non-functional. Such efforts are not required for proteins because they retain their 3-D structure and can still be functional when adsorbed directly on the surface of the nanotube – unlike DNA. The description in D4 does not teach how to attach functional DNA to anchors adsorbed on nanotubes. Also, D4 has not demonstrated that proteins are attached to the anchor – very likely they are just physically adsorbed directly on the surface.

D5

This reference describes pairs or arrays of vertically-aligned carbon nanotubes connected to single strand(s) of DNA through an intermediary metallic material. When the complementary strand of DNA binds to form a double helix, the conductance between the pair of nanotubes is proposed to increase, thus detecting the presence of a DNA target of interest.

The important difference between the present invention and D5 is that the present invention describes *direct* binding of DNA to the nanotubes and uses thereof, while D4 describes *indirect* binding of DNA to the nanotubes through a metallic intermediary. For example, D4 describes DNA bound to gold, with the gold then bound to the nanotubes via thiol-functional groups on the nanotubes. The connections in their proposed uses are of the type nanotubes-gold-DNA, or nanotubes-gold-DNA-gold nanotubes). In contrast, the connections of the present invention are nanotubes-DNA-gold, or nanotubes-DNA-gold-DNA-nanotubes.

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D6

This reference relates to a device for direct reading of the base sequence of an RNA molecule. It describes single nucleotides of each of the four bases (A,G,C, and U) attached to four separate tips (which are carbon nanotubes bound to separate metal probes); the base sequence is read when the appropriate base on one of the nanotube tips is attracted to its complementary base in the RNA molecule bound to a solid substrate, causing distortion of the metal probe which is detected by deflection of a laser beam from the probe.

This device is not relevant to the present invention as it uses only singe nucleotides, not oligonucleotides, attached to carbon nanotubes.

The method of attachment of the nucleotides to the nanotube is not described, other than that nanotubes modified with COO- groups are immersed in an aqueous solution of nucleotides for 2 hours "to effect the chemical modification of nucleotides to the carbon nanotubes". In fact such a process would result simply in the nucleotides physically sticking to the surfaces of the nanotubes, mainly through the bases, which would render them incapable of hydrogen-bonding to their complementary base (and hence they are useless in the device).

Amendments have been proposed in answer to Section VIII of the Written Opinion.

It is respectfully submitted that the objections raised in the Written Opinion now be withdrawn.

Yours faithfully

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nanotubes either side-to-side or end-to-end.

SUMMARY OF THE INVENTION

The inventors have now developed a process capable of linking nanotubes. Importantly, the inventors have developed a process, which allows linkage of nanotubes either side-to-side or end-to-end, thereby dramatically increasing their usefulness. The inventors have also developed a process of physically modifying the walls of nanotubes, while preserving the sp^2 structure of the nanotubes and thus their electronic characteristics. The inventors have also developed a method for locating nanotubes to specific targets. The inventors have also developed techniques which allow DNA patterning on nanotubes as well as the creation of multiple layers of nanoparticles on the surface of nanotubes.

In its broadest aspect, the invention provides a method of chemically attaching nucleic acid molecules to one or more nanotubes. The invention also provides a method of physically attaching nucleic acid molecules to one or more nanotubes. The invention also provides a method of linking these nanotubes. Further, the invention provides a process whereby nanotubes may be directed to specific locations.

Accordingly, in a first aspect, the present invention provides a nanotube with one or more nucleic acid molecule(s) attached thereto.

In a second aspect, the invention provides a method of chemically modifying a nanotube comprising either:

(i) a) chemically attaching at least one linker attached to one or more nucleic acid molecules to an optionally functionalised nanotube, wherein said linker consists wholly or partly of a functional group with the proviso that when the nanotube is functionalised with CO_2H, then the linker is not a primary aliphatic alkyl amine; and

b) synthesising at least two nucleic acid molecules, by sequential addition of nucleotides *in situ*, starting from said one or more nucleic acid molecules; or

(ii) a) chemically attaching at least one linker attached to one or more nucleic acid molecule to an optionally functionalised nanotube, wherein said linker consists wholly or partly of a functional group; and

b) synthesising at least two nucleic acid molecules, by sequential addition of nucleotides *in situ*, starting from said one or more nucleic acid molecules; or

(iii)a) chemically attaching at least one linker to an optionally functionalised nanotube, wherein said linker consists wholly or partly of a functional group; and

b) attaching one or more nucleic acid molecules to said optionally functionalised nanotube via said functional group on said linker; or

(iv) (a) chemically attaching at least one linker to an optionally functionalised nanotube, wherein said linker consists wholly or partly of a functional group; and

b) synthesising one or more nucleic acid molecules, by sequential addition of nucleotides *in situ*, starting from said functional group on said linker.

In a third aspect, the invention provides a method of chemically modifying a nanotube comprising either:

(i) a) photochemically attaching at least one linker attached to one or more nucleic acid molecules to an optionally functionalised nanotube, wherein said linker consists wholly or partly of a functional group; and

b) synthesising at least two nucleic acid molecules by sequential addition of nucleotides *in situ*, starting from said one or more nucleic acid molecules; or

(ii) a) photochemically attaching at least one linker to an optionally functionalised nanotube, wherein the linker consists wholly or partly of a functional group; and

b) attaching one or more nucleic acid molecules to said optionally functionalised nanotube via said functional group on said linker; or

(iii) a) photochemically attaching at least one linker to an optionally functionalised nanotube, wherein the linker consists wholly or partly of a functional group; and

b) synthesising one or more nucleic acid molecules, by sequential addition of nucleotides *in situ*, starting from said functional group on said linker.

In a fourth aspect, the invention provides a method of physically modifying a nanotube comprising either:

(i) a) physically adsorbing at least one anchor attached to one or more nucleic acid molecules to the surface of an optionally functionalised nanotube, wherein said anchor consists wholly or partly of a functional group;

b) synthesising at least two nucleic acid molecules by sequential addition of nucleotides *in situ*, starting from said functional group on said anchor; or

(ii) a) physically adsorbing at least one anchor to the surface of an optionally functionalised nanotube, wherein said anchor consists wholly or partly of a functional group; and

b) chemically attaching one or more nucleic acid molecules to said functional group on said anchor adsorbed on the optionally functionalised nanotube; or

(iii)a) physically adsorbing at least one anchor attached to one or more nucleic acid molecules to the surface of an optionally functionalised nanotube, wherein said anchor consists wholly or partly of a functional group;

b) synthesising one or more nucleic acid molecules, by sequential addition of nucleotides *in situ*, starting from said functional group on said anchor.

In a fifth aspect, the invention provides a plurality of linked nanotubes.

In a sixth aspect, the present invention provides a method of linking nanotubes comprising the steps of:

a) attaching a first nucleic acid molecule of a first base sequence to a first optionally functionalised nanotube; and

b) hybridizing the first nucleic acid molecule with

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A nanotube with one or more nucleic acid molecule(s) attached thereto.

2. A method of chemically modifying a nanotube comprising either:

(i) a) chemically attaching at least one linker attached to one or more nucleic acid molecules to an optionally functionalised nanotube, wherein said linker consists wholly or partly of a functional group with the proviso that when the nanotube is functionalised with CO_2H, then the linker is not a primary aliphatic alkyl amine; and

b) synthesising at least two nucleic acid molecules, by sequential addition of nucleotides *in situ*, starting from said one or more nucleic acid molecules; or

(ii) a) chemically attaching at least one linker attached to one or more nucleic acid molecule to an optionally functionalised nanotube, wherein said linker consists wholly or partly of a functional group; and

b) synthesising at least two nucleic acid molecules, by sequential addition of nucleotides *in situ*, starting from said one or more nucleic acid molecules; or

(iii)a) chemically attaching at least one linker to an optionally functionalised nanotube, wherein said linker consists wholly or partly of a functional group; and

b) attaching one or more nucleic acid molecules to said optionally functionalised nanotube via said functional group on said linker; or

(iv) a) chemically attaching at least one linker to an optionally functionalised nanotube, wherein said linker consists wholly or partly of a functional group; and

b) synthesising one or more nucleic acid molecules, by sequential addition of nucleotides *in situ*, starting from said functional group on said linker.

3. A method of chemically modifying a nanotube comprising either:

(i) a) photochemically attaching at least one linker attached to one or more nucleic acid molecules to an optionally functionalised nanotube, wherein said linker consists wholly or partly of a functional group; and

(b) synthesising at least two nucleic acid molecules by sequential addition of nucleotides *in situ*, starting from said one or more nucleic acid molecules; or

(ii) a) photochemically attaching at least one linker to an optionally functionalised nanotube, wherein the linker consists wholly or partly of a functional group; and

b) attaching one or more nucleic acid molecules to said optionally functionalised nanotube via said functional group on said linker; or

(iii)a) photochemically attaching at least one linker to an optionally functionalised nanotube, wherein the linker consists wholly or partly of a functional group; and

b) synthesising one or more nucleic acid molecules, by sequential addition of nucleotides *in situ*, starting from said functional group on said linker.

4. A method of physically modifying a nanotube comprising either:

(i) (a) physically adsorbing at least one anchor attached to one or more nucleic acid molecules to the surface of an optionally functionalised nanotube, wherein said anchor consists wholly or partly of a functional group;

b) synthesising at least two nucleic acid molecules by sequential addition of nucleotides *in situ*, starting from said functional group on said anchor; or

(ii) a) physically adsorbing at least one anchor to the surface of an optionally functionalised nanotube, wherein said anchor consists wholly or partly of a

functional group; and

b) chemically attaching one or more nucleic acid molecules to said functional group on said anchor adsorbed on the optionally functionalised nanotube; or

(iii)a) physically adsorbing at least one anchor attached to one or more nucleic acid molecules to the surface of an optionally functionalised nanotube, wherein said anchor consists wholly or partly of a functional group;

b) synthesising one or more nucleic acid molecules, by sequential addition of nucleotides *in situ*, starting from said functional group on said anchor.

5. A method of linking nanotubes comprising the steps of:

a) attaching a first nucleic acid molecule of a first base sequence to a first optionally functionalised nanotube; and

b) hybridising the first nucleic acid molecule with a second nucleic acid molecule of a second base sequence attached on a second optionally functionalised nanotube, wherein the base sequence of the second nucleic acid molecule is substantially complementary to the base sequence of the first nucleic acid molecule.

6. A method of linking nanotubes comprising the steps of:

a) attaching a first nucleic acid molecule of a first base sequence to optionally functionalised nanotubes; and

b) hybridising the first nucleic acid molecule with a second nucleic acid molecule which comprises a base sequence substantially complementary to the first base sequence and further comprises a second or a third base sequence which is/are not complementary to the first base sequence, but is/are complementary to each